(SiO_2, 17 mm i.d., 3 cm, hexane), and the green band on the column yielded a small amount of product. Its ^1H NMR spectrum was identical with that of **11** obtained by bromination of **2**.

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Registry No. **2**, 4670-86-4; **2**·1,3,5-trinitrobenzene, 124581-61-9; **2**·2,4,7-trinitro-9-fluorenone, 124581-62-0; **3**, 22674-47-1; **4**, 74067-57-5; **8**, 15364-55-3; **9**, 793-40-8; **10**, 97733-14-7; **11**, 124581-63-1; **12**, 124581-64-2; **13**, 124581-65-3; **14**, 124581-66-4; **15**, 124581-67-5; **16**, 124581-68-6; benzo[*a*]fluoroanthene, 203-33-8.

Secondary α-Deuterium Isotope Effects in the Formation of Imines

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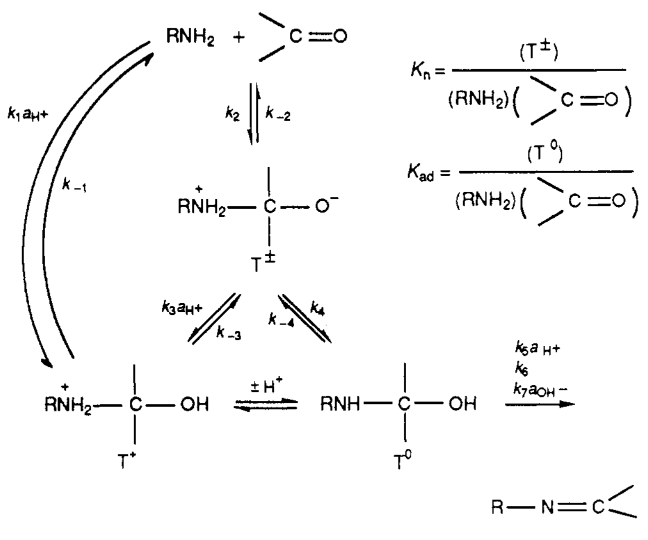
Kinetic α-deuterium isotope effects, k_D/k_H, for hydronium ion catalyzed addition ($k_1a_{H^+}$) of semicarbazide ($pK_a = 3.86$) to *m*-bromobenzaldehyde and *p*-nitrobenzaldehyde, and of methoxyamine ($pK_a = 4.73$) and hydroxylamine ($pK_a = 6.15$) to *m*-bromobenzaldehyde, are near 1.14 and independent of the nature of both the nucleophile and the benzaldehyde employed. The corresponding values for the uncatalyzed reaction (k_2) of *m*-bromobenzaldehyde with several nucleophiles decrease from 1.30 to 1.16 when the nucleophile is changed from semicarbazide to hydroxylamine. For the addition of semicarbazide, k_D/k_H decreases from 1.14 to 1.11 as the benzaldehyde is varied from *m*-bromobenzaldehyde to *p*-nitrobenzaldehyde. Values of k_D/k_H for the hydronium ion catalyzed process, which converts the zwitterionic intermediate $T^{\pm}$ to T^+ ($K_nk_3a_{H^+}$), are near 1.21 for the reaction of several nucleophiles with all the benzaldehydes studied. The isotope effect observed for the uncatalyzed formation of the carbinolamine intermediate T^0 (K_nk_4) suggests a dependence on the nature of the nucleophile and the benzaldehyde. These results indicate that the extent of carbon–nitrogen bond formation in the transition state for addition of the nucleophiles to the carbonyl group is a function of the reactivity of the reagents and the catalysts.

The reaction of nitrogen nucleophiles with carbonyl compounds occurs in two general stages: formation of a carbinolamine addition intermediate followed by carbinolamine dehydration to yield the imine product.[2-3] Carbinolamine formation occurs by at least three separate routes[4-6] as shown in Scheme I, concerted addition of amine and protonation of the carbonyl oxygen atom ($k_1a_{H^+}$), a stepwise route involving trapping by proton transfer of a dipolar compound formed by addition of amine to the carbonyl group (k_2 followed by $K_nk_3a_{H^+}$ or K_nk_4), and a "spectator" mechanism involving preassociation of acid catalyst with the carbonyl oxygen.[7] Subsequent carbinolamine dehydration may occur via acid-catalyzed, pH-independent, and base-catalyzed pathways ($k_5a_{H^+}$, k_6, $k_7a_{OH^-}$).

Consequently, by appropriate choice of experimental conditions, a single set of compounds can be employed to probe the transition-state structure for several different steps. As a specific set of reactants, we have chosen substituted benzaldehydes as the carbonyl component and semicarbazide, methoxyamine, phenylhydrazine, and hydroxylamine as the amines. This permits study of the effect of variation of both substrate and nucleophile on the transition-state structure. Moreover, since the addition step for these reactants can be either pH-independent or hydronium ion catalyzed, the relationship between catalysis and transition-state structures may also be probed. This paper reports α-deuterium isotope effects for these reactions as a function of the structure of carbonyl component, amine, and acid catalysis.

Scheme I



Experimental Section

Materials. Analytical grade reagent inorganic salts were used without further purification. Glass-distilled water was used

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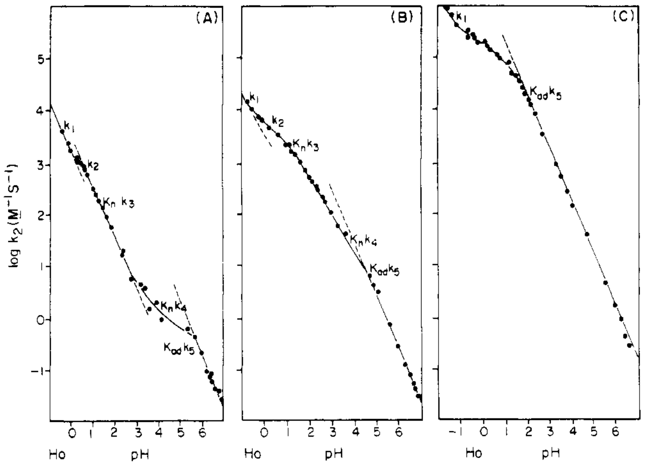

Figure 1. Plots of the logarithms of the second-order rate constants, $k_{obs}°$ (corrected for carbinolamine accumulation where necessary and extrapolated to zero buffer concentration), for the reaction of *m*-bromobenzaldehyde with semicarbazide (A), methoxyamine (B), and hydroxylamine (C) plotted as a function of pH or H_0. All measurements were made in aqueous solutions at 25.0 °C and an ionic strength of 1.0. The solid curves are theoretical fits based on eq 1–3 and the rate constants collected in Table I. The values of H_0 in aqueous solutions used at the highest acid concentration were taken from Paul, M. A.; Long, F. A. *Chem. Rev.* **1957**, *57*, 1. Sadek, H.; Elamayem, M. S. A.; Sidahmed, I. M. *Suom. Kemistil* **1966**, *39*, 225.

throughout. Semicarbazide hydrochloride, hydroxylamine hydrochloride, and methoxyamine hydrochloride were recrystallized prior to use. *m*-Bromobenzaldehyde-α-d_1[8] and *p*-nitrobenzaldehyde-α-d_1[9] were prepared as previously described. The ^{1}H NMR spectra (Varian A-60 spectrometer) of neat samples of all deuterated compounds indicated an isotopic purity of at least 98% in all cases.

Kinetic measurements were carried out spectrophotometrically at 25.0 °C with the aid of a Zeiss PMQ II spectrophotometer equipped with a thermostated cell holder. In all cases, a sufficient excess of nucleophilic reagent was employed to insure pseudo-first-order rate behavior. First-order rate constants were evaluated from slopes of plots of log ($OD_\infty - OD_t$) against time in the usual manner. Second-order rate constants were obtained by dividing the first-order rate constants by the appropriate concentration of nucleophilic reagent in the reactive, free-base form. In the region in which carbinolamine formation is principally rate-determining, rate constants were corrected for the influence of the rate of carbinolamine dehydration, as described by Sayer and Jencks.[10] Catalytic (third-order) rate constants were evaluated from the slopes of plots of second-order rate constants against the concentration of the catalyst.

All kinetic experiments were carried out at 25.0 ± 0.1 °C in aqueous solution at an ionic strength of 1.00, maintained with KCl, in the presence of 2.0×10^{-4} M EDTA. Values of pH were recorded with a Micronal Model B-275 pHmeter equipped with a glass electrode. Concentrations of the nucleophile in the free-base form and of undissociated carboxylic acid were calculated employing the Henderson–Hasselbalch equation and appropriate values of pK_a (pK_a: semicarbazide = 3.86; methoxyamine = 4.73; and hydroxylamine = 6.15).[4] The determined values of k_{exp} are reproducible within an experimental error of 2%.

The kinetic procedure utilized to determine the isotopic effects has been previously described.[9]

Results

The experimental pH–rate profiles for the reactions of *m*-bromobenzaldehyde with semicarbazide (A), methoxyamine (B), and hydroxylamine (C) are presented in Figure 1. Steady-state treatment of the mechanism in Scheme I yields the following equations for product formation in the region where carbinolamine formation is rate-determining:

$$k_{obs}° = \frac{k_{ad}K_{ad}°k_5 a_{H^+}}{k_{ad}° + K_{ad}k_5 a_{H^+}} \tag{1}$$

$$k_{ad}° = k_1 a_{H^+} + \frac{k_2(K_n k_3 a_{H^+} + K_n k_4)}{k_2 + K_n k_3 a_{H^+} + K_n k_4} \tag{2}$$

The equation for the rate-determining carbinolamine dehydration is:

$$k_{deh}° = K_{ad}k_5 a_{H^+} \tag{3}$$

The fit of experimental points to the theoretical curves, in Figure 1, calculated by employing the rate laws in eq 1–3, together with the rate constant in Table I, is acceptable in all three cases.

The pH–rate profiles for the reaction of *m*-bromobenzaldehyde with semicarbazide and with methoxyamine show the break in the pH range 4–6 characteristic of a transition from rate-determining carbinolamine formation at more acidic pH to carbinolamine dehydration at more basic pH. In both cases, the pH–rate profiles also exhibit a second break under more acidic conditions. The second break presumably reflects a transition from acid-catalyzed stepwise formation of carbinolamine ($K_n k_3 a_{H^+}$) at pH > 1–2, to uncatalyzed addition of amine (k_2) between about pH 0 and 1 and, finally, to concerted addition mechanism ($k_1 a_{H^+}$) under even more acidic conditions.[4,5] The pH–rate profile for the reaction of *m*-bromobenzaldehyde with hydroxylamine, a very strong nucleophile, shows only a single break in the pH range 1–2.

Under conditions in which formation of the carbinolamine intermediate is rate-determining, the second-order rate constants for the reaction of *m*-bromobenzaldehyde with semicarbazide are a sensitive function of the nature and concentration of the carboxylic acid/carboxylate buffer employed to maintain constant pH. Measurements of the catalytic effect as a function of the ratio of acidic and basic forms of the buffer established that, within experimental error, the catalysis is of the general acid type. Thus, the plots of the second-order rate constants against the con-

Table I. Rate and Equilibrium Constants for the Reaction of *m*-Bromobenzaldehyde with Semicarbazide, Methoxyamine, and Hydroxylamine in Aqueous Solution at 25.0 °C and an Ionic Strength of 1.0

	semicarbazide	methoxyamine	hydroxylamine
k_1, mol^{-2} dm^6 s^{-1}	1.7×10^3	2.0×10^3	1.0×10^4
k_2, mol^{-1} dm^3 s^{-1}	9.0×10^2	3.0×10^3	2.0×10^5
$K_n k_3$, mol^{-2} dm^6 s^{-1}	3.6×10^3	8.0×10^4	–
$K_n k_4$, mol^{-1} dm^3 s^{-1}	1.0	1.0×10^1	–
$K_{ad}k_5$, mol^{-2} dm^6 s^{-1}	2.0×10^5	2.5×10^5	1.8×10^6

Table II. Catalytic Constants for Several Acids for the Reaction of *m*-Bromobenzaldehyde and Semicarbazide in Aqueous Solution at 25.0 °C and an Ionic Strength of 1.0

catalyst	pK_a	pH	conc, M	k_{cat}, mol^{-2} dm^6 s^{-1}
H_3O^+	−1.74	0.25–1.5		3.6×10^3
$NCCH_2CO_2H$	2.45	2.34	0.020–0.25	4.6×10^2
$ClCH_2CO_2H$	2.90	2.36	0.020–0.25	3.4×10^2
$ClCH_2CO_2H$	2.90	2.70	0.020–0.25	3.5×10^2
$ClCH_2CO_2H$	2.90	3.10	0.020–0.25	3.2×10^2
$BrCH_2CH_2CO_2H$	4.00	3.36	0.020–0.25	2.9×10^2
HCO_2H	3.75	3.60	0.020–0.25	2.5×10^2
CH_3CO_2H	4.76	4.10	0.020–0.25	2.2×10^2

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Table III. pH or H_o Used for the Determination of Kinetic α-Deuterium Isotope Effects, Expressed in k_D/k_H, for the Addition of Nucleophiles to Substituted Benzaldehydes

reagents	H_o	pH	rate constant
semicarbazide and *m*-bromobenzaldehyde	−0.47		k_1
	−0.55		k_2
semicarbazide and *p*-nitrobenzaldehyde	−1.47		k_1
	−0.03		k_2
		2.01	K_nk_3
		4.55	K_nk_4
methoxyamine and *m*-bromobenzaldehyde	−1.01		k_1
	0.20		k_2
		2.00	K_nk_3
		4.00	K_nk_4
hydroxylamine and *m*-bromobenzaldehyde	−1.47		k_1
	−0.03		k_2

centration of the acidic component of the buffer yield straight lines with equal slopes, regardless of the buffer composition. Catalytic constants were obtained directly from these slopes (Table II). These results are consistent with previously established catalytic patterns for closely related reactions.[4,11] Correlation of the catalytic constants with the pK_a of the carboxylic acids, in Brønsted plots, gives a value of 0.12 for the Brønsted α value.

Catalysis by the carboxylic acid/carboxylate buffer was also observed for the reaction of *m*-bromobenzaldehyde with methoxyamine. However, it was not possible to calculate values of catalytic constants due to the rapidity of the subsequent dehydration reaction. The same difficulty has been encountered in a similar reaction.[5] For the reaction of *m*-bromobenzaldehyde with hydroxylamine, catalysis by the carboxylic acid/carboxylate buffers was not observed since the rate-determining step is dehydration of the carbinolamine in this pH range.

Measurement of isotope effects on the individual rate constants for addition of amines to benzaldehydes is complicated by difficulties in achieving experimental conditions under which just one rate constant dominates the kinetics. pH values were chosen so as to minimize these complications (Table III).

The measured α-deuterium isotope effects, expressed as k_D/k_H, on the various rate constants for reactions of several substituted benzaldehydes and nitrogen nucleophiles are collected in Table IV.

Discussion

The secondary kinetic isotope effect has been used to provide a knowledge of the position of the transition state relative to the progress of a reaction.[12]

In this work, we determined pH–rate profiles for the reactions of *m*-bromobenzaldehyde with semicarbazide, methoxyamine, and hydroxylamine (Figure 1). Literature[4] pH–rate profiles were available for the reactions of *p*-nitrobenzaldehyde and *p*-methoxybenzaldehyde with semicarbazide. Based on these five pH–rate profiles, we determined the secondary kinetic α-deuterium isotope effect for all the steps of the formation of the corresponding carbinolamines. The values of k_D/k_H are shown in Table IV.

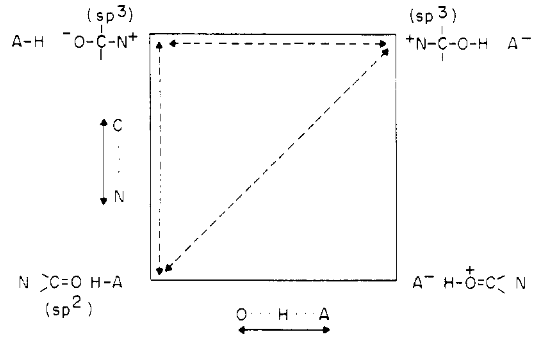
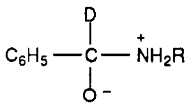
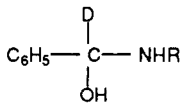


Figure 2. Schematic representation of a contour diagram for the complex general acid catalysis, where the movement of the proton is shown on the horizontal axis. For the concomitant mechanism, the transition state of the rate-determining step occurs in the central part of the scheme. For the stepwise mechanism, the reaction proceeds by the borderlines of the scheme and involves different intermediate transition states.[15]

The isotope effect on the equilibrium constant for carbinolamine formation was determined previously[12] as 1.36 ± 0.02. This value provides a basis for the interpretation of kinetic isotope effects for the same reaction in terms of the degree of carbon–nitrogen bond formation in the transition state, i.e., kinetic isotope effect should increase from 1.00 to 1.36 ± 0.02 as carbon–nitrogen bond formation becomes increasingly complete. This conclusion is based on the reasonable assumption[12] that there is little or no difference in the isotopic fractionation factors between the species:


$$C_6H_5-\overset{\displaystyle D}{\underset{\displaystyle O^-}{C}}-\overset{+}{N}H_2R$$

and


$$C_6H_5-\overset{\displaystyle D}{\underset{\displaystyle OH}{C}}-NHR$$

The extent of carbonyl carbon–nucleophile bond formation can be calculated by using eq 4.

$$B = |(k_H/k_D) - 1|/|(K_H/K_D) - 1| \tag{4}$$

The values of k_D/k_H for the pH region corresponding to k_1 as the rate-determining step is approximately constant and equal to 1.14 ± 0.03 for the reactions of semicarbazide with *m*-bromo- and *p*-nitrobenzaldehydes, and methoxyamine with *m*-bromobenzaldehyde. This isotopic value corresponds to $B = 0.39$. These values suggest that the transition state more nearly resembles the reactants.

The secondary kinetic isotope effects, k_D/k_H, measured in the pH range corresponding to k_1 as the rate-determining step, is independent of the basicity of parent amines (semicarbazide and methoxiamine) and of the reactivity of the aldehydes. This observation may be interpreted using a contour diagram as proposed by Jencks[14] in the study of the mechanism of acid–base catalysis in complex reactions (Figure 2).

The transition state for the concomitant mechanism, in which the rate-determining step is k_1, occurs in the central region of the diagram, in which C–N bond formation and the proton transfer occur simultaneously. The value of B may reflect the extent of C–N bond formation in the transition state, and the differences between the transition states of the studied reaction considered in routes 1, 2, and 3 (Figure 3) are so small that the secondary isotopic effect is not significantly affected.

In the pH range where k_2, the uncatalyzed formation of the zwiterionic intermediate $T^{\pm}$, is the rate determining step, the values of k_D/k_H for *m*-bromobenzaldehyde decrease from 1.20 to 1.16 as the nucleophile is changed from

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Table IV. Kinetic α-Deuterium Isotope Effects, Expressed as k_D/k_H for the Addition of Several Nucleophiles to Substituted Benzaldehydes at 25.0 °C[b]

	p-methoxy	hydrogen	*m*-bromo	*p*-nitro
Semicarbazide (pK_a = 3.86)				
k_1	-	-	1.144 ± 0.029	1.112 ± 0.014
k_2	-	-	1.199 ± 0.014	1.109 ± 0.022
K_nk_3	1.216 ± 0.06[a]	1.207 ± 0.007[a]	1.218 ± 0.004	1.185 ± 0.006
K_nk_4	1.310 ± 0.004[a]	1.312 ± 0.004[a]	1.285 ± 0.004	1.179 ± 0.007
Methoxyamine (pK_a = 4.73)				
k_1	-	-	1.172 ± 0.013	-
k_2	-	-	1.174 ± 0.014	-
K_nk_3	-	-	1.220 ± 0.019	-
K_nk_4	-	-	1.233 ± 0.028	-
Phenylhydrazine (pK_a = 5.30)				
k_1	-	-	-	-
k_2	-	-	-	-
K_nk_3	1.208 ± 0.010[a]	1.218 ± 0.007[a]	-	1.126 ± 0.007[a]
K_nk_4	1.276 ± 0.011[a]	1.218 ± 0.007[a]	-	1.165 ± 0.006[a]
Hydroxylamine (pK_a = 6.15)				
k_1	-	-	1.096 ± 0.003	-
k_2	-	-	1.158 ± 0.011	-

[a] Values for ref 12. [b] Errors are given as the standard deviation of the mean of five determinations.

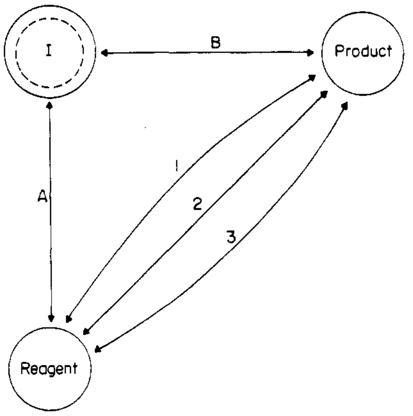

Figure 3. Energy diagram of the reaction coordinate to show that a reaction that needs two process A and B may occur in two steps through an intermediate with a significant life time, *I*, or through a concomitant mechanism, 1, 2, or 3, in which the transition states have different degrees of similarity with the structure of the intermediate.

semicarbazide (less basic) to hydroxylamine (more basic). The corresponding *B* values decrease from 0.56 to 0.44. Also on going from *m*-bromobenzaldehyde (less reactive) to *p*-nitrobenzaldehyde (more reactive) the values of k_D/k_H for the attack of semicarbazide decrease from 1.20 to 1.11, corresponding to a decrease in *B* from 0.56 to 0.31. The variation in the values of k_D/k_H, in uncatalyzed formation of the zwiterionic intermediate $T^{\pm}$, as a function of the nature of nucleophile and aldehydes, may be interpreted using the scheme in Figure 3. The transition state for the formation of $T^{\pm}$ presents different degrees of formation, or rupture, of the C–N bond as a function of the reagents. For analogous amines, with the more basic (hydroxylamine), the transition state will have smaller degree of formation of C–N bond than with the less basic (semicarbazide). So, the transition state will be closer to the reagents in the reaction coordinate. Similar considerations may be made concerning the variation of the reactivity of the aldehyde with semicarbazide. The results are in accord with the Marcus's arguments.[16]

The values of k_D/k_H for the pH region corresponding to K_nk_3 as the rate-determining step are approximately constant and equal to 1.21 ± 0.02 (B = 0.58), for the reaction of semicarbazide with *p*-methoxybenzaldehyde, benzaldehyde, *m*-bromobenzaldehyde, and *p*-nitrobenzaldehyde. One may note that in K_nk_3 there are two steps, the attack of the nucleophile on the aldehyde (partial formation on C–N bond in the transition state) with formation of the intermediate $T^{\pm}$, followed by a proton transfer from the catalyst to the intermediate $T^{\pm}$ leading the formation of the intermediate T^+. Then, one may expect that the isotope effect k_D/k_H measured for K_nk_3 would be similar to that measured for the k_2 step. The value of 1.21 measured for K_n/k_3 is accord with this expectation, since its value is close to the maximum value of k_D/k_H for k_2 (1.20). It is possible to explain the constancy of the value of k_D/k_H, independent of the nature of the nucleophile or of the aldehyde, since k_2 must be very fast in this pH region and the transition state for rate-determining step occurs along the reaction coordinate corresponding to the proton transfer, from the hydronium ion to the intermediate $T^{\pm}$, previously formed very quickly (k_2) (see Figure 2). The observed isotopic effect corresponds to the formation of the C–N bond that occurs in the previous step.

As the reactivity of the carbonyl compound is increased, the values of k_D/k_H for the pH region corresponding to K_nk_4 decrease from 1.31 to 1.18, in the reaction with semicarbazide, and from 1.28 to 1.16 in the reaction with phenylhydrazine. This corresponds respectively to decrease in the value of *B* from 0.86 to 0.50 and from 0.78 to 0.44. For the same aldehyde one also observes that the values of k_D/k_H decrease with increasing reactivity of the nucleophile. The values of the rate constants in the pH region corresponding to K_nk_4 (pH ≅ 4.5 acetic acid/acetate buffer) were influenced by catalytic species: hydronium ion, acetic acid, and water. In this case, the analysis of the isotopic effect is hampered by the magnitude of the corrections necessary for calculation of the rate constant due only to water.

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